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Plant stress detection by remote measurement of fluorescence

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Chlorophyll fluorescence of mature lemon trees was measured with a Fraunhofer line discriminator (FLD). An increase in fluorescence was correlated with plant water stress as measured by stomatal resistance and twig water potential.

I. Introduction

Plant growth and development are based on the capture and transformation of electromagnetic radiation by chlorophyll. The efficiency of photosynthesis depends upon the presence of sufficient amounts of water, mineral nutrients, carbon dioxide, and light; and it can be regulated by any factor which interferes with the availability or mixture of those items. Plant damage by pollutants, water stress, or pathogens usually results in a reduced rate of photosynthesis. If damage is severe, visible symptoms may be evident on the leaves or stems. However, a decreased photosynthetic rate may not be visually evident but can result in reduced production that may be recognized only at harvest or when ameliorative measures no longer help. Measurement of the depression of photosynthetic rates is, therefore, an important criterion in the evaluation of pollutant effects, water sufficiency, and other stress conditions on plants.

When photosynthetic rates are decreased without altering or decreasing the chlorophyll content, radiant energy absorbed by chlorophyll must be dissipated by a mechanism other than the fixation of carbon; fluorescence is one means of releasing excess energy from chlorophyll. Chlorophyll fluorescence occurs in the visible spectrum, and although it represents measurable quantities of light, it is small compared with the amount of energy reflected from the leaf surface. Therefore, under conditions of daylight, plant fluorescence is not observed without the assistance of sophisticated detecting equipment. A device called the Fraunhofer line discriminator (FLD) was built by the U.S. Geological Survey to examine fluorescent phenomena that are used in identifying ore deposits,[1] industrial and natural wastes,[2] contamination of water systems by fluorescent materials,[3] and plant fluorescence.[4] In brief, the Fraunhofer line depth principle[5] involves using the sun as an excitation source. When a target is exposed to the sun, the infilling of a Fraunhofer line (increased radiation at the central wavelength of the Fraunhofer line) can be attributed to fluorescence. The effects of variations in solar radiation are eliminated by taking a ratio of the radiance in the line center to that of the continuum adjacent to the Fraunhofer line. Three Fraunhofer lines, 486.1, 589.0, and 656.3 nm, are selectable with the current FLD. The 656.3-nm Fraunhofer line is used to measure chlorophyll fluorescence.

The experiment described in this paper was designed to determine if a correlation existed between plant stress, as measured by typical physiological parameters, and changes in plant fluorescence as measured by the FLD. Since the availability of water regulates stomatal aperture, water shortage was chosen as a nondestructive method of applying stress to the plants. Under stressed conditions, one way that plants conserve water is by limiting the size of the stomata; but since CO_2 enters through the same portal, a smaller opening may also reduce the available CO_2 and hence the photosynthetic rate. Absorbed energy not used in carbon fixation becomes excess and must be reradiated. All or part of this excess energy could be dissipated by fluorescence of the chlorophyll.

II. Methods

An experiment was conducted at the University of Arizona Citrus Experiment Farm in Tempe, Arizona. Thirteen mature lemon trees were selected for study. Irrigation water was withheld from all the trees for three

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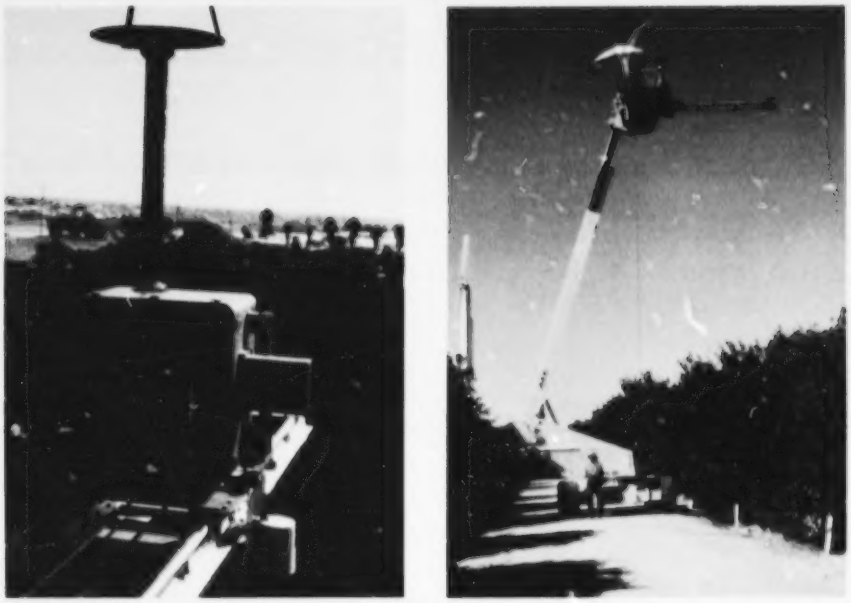

(a) (b)

Fig. 1. Fraunhofer line discriminator unit (a) above a citrus tree and (b) a side view.

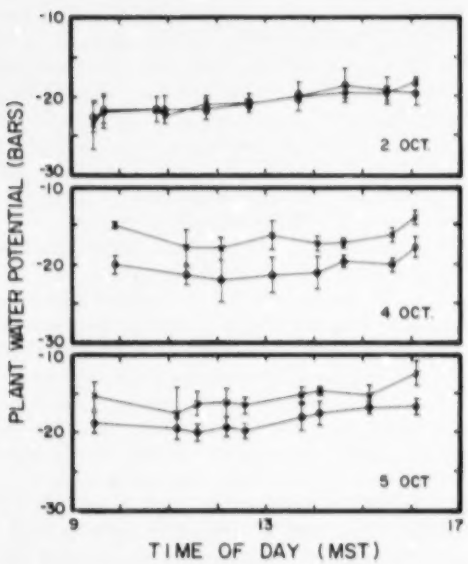

Fig. 2. Daytime measurements of plant water potential. Mean values for irrigated trees are indicated by × and for nonirrigated trees by O. Standard deviations are indicated by vertical lines.

weeks prior to the study in an attempt to induce water stress. However, a 1.25-cm rain occurred one week before the measurement dates (3, 4, and 5 Oct. 1977), which decreased the severity of water stress. At the beginning of the experiment, alternate groups of trees were irrigated to create a condition of stress in some and nonstress in others. The treatment groups were trees 1 and 2 (wet 1); 3 and 4 (dry 1); 5, 6, 7, and 8 (wet 2); 9, 10, and 11 (dry 2); 12 and 13 (wet 3). On the fourth day of the experiment (6 Oct.) a heavy rain fell upon the entire orchard, and the study was terminated.

The Fraunhofer line discriminator (FLD) measured the chlorophyll fluorescence of these trees from a platform 12 m (40 ft) above the ground (Fig. 1). The FLD was mounted on a table which rode on a track. As it traversed to the end of the track, the sensor scanned back and forth across the top of the canopy collecting data 36 times each second (1080 measurements per tree per traverse). The measurements from each tree were averaged, and the treatment means represent the values and the standard deviations between trees in each group.

Water potential measurements[6] were made on twigs clipped at chest height at four points (two in the sun and two in the shade) around four trees, two not irrigated (3 and 4) and two irrigated (5 and 6). Since no statistical differences were found between sun and shade measurements, mean values of the data are presented. Diffusive resistance[7] of the stomates was determined by measuring the rate of water vapor evolution in a Lambda[8] porometer. Samples for diffusive resistance were taken at chest height at eight points around the perimeter of the same trees.

III. Results and Discussions

Twig water potential measurements indicated that the trees were under similar stress before irrigation (Fig. 2, see 2 Oct.). Afterward, the irrigated trees were clearly less stressed than the nonirrigated trees (Fig. 2, see 4 and 5 Oct.).

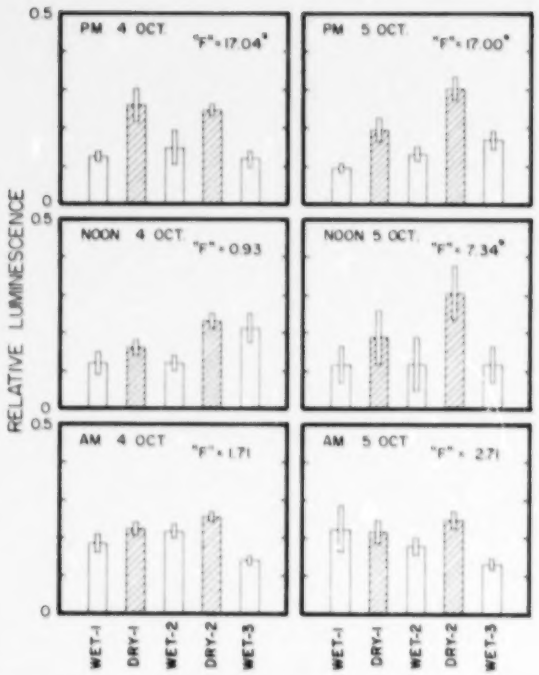

Fig. 3. Luminescence histogram of water stressed (dry) and nonstressed (wet) citrus trees. Small bars indicate error limits. F is the F-statistic, and * indicates significant differences at the 0.05 level in the canopy fluorescence of irrigated and nonirrigated trees.

Stomatal diffusive resistance values of the irrigated and nonirrigated trees are given in Table I. Using this measurement, a separation of nonstressed and stressed trees is evident in the afternoon samplings; however, in the morning and at noon, sufficient water stress had not developed to show significant differences between the irrigated and nonirrigated trees.

Data from the Fraunhofer line discriminator (FLD) are shown in Fig. 3. The F ratio in an analysis of variance indicated no difference in the mean fluorescence of the irrigated and nonirrigated trees in the prenoon measurements. Differences in fluorescence were evident in the afternoons. The most significant separation of values occurred in the latest samplings (i.e., 1500 h). Measurements of stomatal resistance indicated that severe water stress in the nonirrigated plants occurred in the afternoons. The correlation between stomatal resistance and luminescence was greatest at 1500 h, with a value of 0.8 ($P \leq 0.05$).

The successful demonstration of different fluorescent patterns between irrigated and nonirrigated trees suggests the possibility of using the FLD to measure plant fluorescence as a means of identifying areas of stress in crop or field plants.

The most important feature of this measurement is that it relates to plant water stress even in the absence of visible signs. Indeed, fluorescence measurements were as sensitive in evaluating plant water stress as stomatal resistance and water potential measurements. Tucker *et al.*[9] observed another spectral change which

Table I. Leaf Diffusion Resistance (R) of Irrigated and Nonirrigated Lemon Trees[a]

Time	Date	Nonirrigated R (sec/cm)	S.D.	Irrigated R (sec/cm)	S.D.
1410–1440	4 Oct	9.6	4.5	3.5	1.2
0908–0920	5 Oct	4.6	2.0	4.5	1.8
1200–1245	5 Oct	8.7	4.5	5.7	1.9
1500–1545	5 Oct	13.4	3.9	6.8	2.2

[a] Values are means of sixteen readings taken in Oct. 1977.

was apparently associated with water stress. In times of low rainfall a perceptable decrease in the infrared/red ratio of reflected radiation from a wheat field was observed. They postulated that this probably represented a transient reduction in leaf chlorophyll density. Although no chlorophyll or plant stress measurements were made, their interpretation appears justified because at the same time the photographic infrared radiance, which is proportional to the leaf area index, was unaltered. In our studies the transient nature of this fluorescent change (midday vs afternoon) suggested that changes in chlorophyll content were not necessarily responsible. Also, a correlation between chlorophyll fluorescence and resistance was found. Nevertheless, transient changes in leaf chlorophyll content would have shown up in a similar pattern, and perhaps the FLD would be complementary to the reflectance measurements in some cases.

The results of this study confirmed a relationship between plant stress and measurable fluorescence and suggest that future studies should incorporate FLD measurements. If the FLD were operated from an aircraft or space platform, the amount of data that could be obtained would be much greater than that could be collected by a group of technicians measuring individual leaves and stems. We propose that plant fluorescence measurements may prove useful in identifying conditions which have interfered with crop photosynthesis.

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